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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN NO. 88.
MILTON WHITNEY, Chief.

SOME ORGANIC SOIL CONSTITUENTS.

BY

EDMUND C. SHOREY,
SCIENTIST IN SOIL FERTILITY INVESTIGATIONS.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS,
Washington, D. C., July 16, 1912.

SIR: I have the honor to transmit herewith the manuscript of a scientific paper entitled "Some Organic Soil Constituents," by Dr. Edmund C. Shorey, of this bureau. This article is the result of an extensive study of a number of soils and reports the further isolation and identification of 14 definite organic compounds from soils, showing the great complexity of the organic matter, and makes clear its chemical nature and suggests their origin in the soil.

The investigation throws considerable additional light upon the questions of soil fertility, and I have, therefore, the honor to recommend that the article be published as Bulletin No. 88 of the Bureau of Soils.

Respectfully,

MILTON WHITNEY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

2

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CONTENTS.

	Page.
Introduction.....	5
Organic acids.....	6
Oxalic acid.....	7
Succinic acid.....	10
Saccharic acid.....	11
Acrylic acid.....	12
Organic bases.....	14
Lysine.....	14
Adenine.....	16
Choline.....	17
Trimethylamine.....	18
Aldehydes.....	19
Salicylic aldehyde.....	20
Unidentified aldehyde.....	20
Carbohydrates.....	21
Mannite.....	21
Rhamnose.....	22
Organic sulphur compounds.....	24
Sulphur.....	25
Trithiobenzaldehyde.....	25
Organic phosphorus compounds.....	27
Nucleic acid.....	29
General methods.....	30
Alkaline extraction.....	31
Unification of methods.....	32
Precipitation with metallic salts.....	33
Precipitation with phosphotungstic acid.....	34
Calcium sulphate contamination.....	34
Calcareous soils.....	35
Changes in organic compounds.....	35
Conclusion.....	36
Summary.....	41

ILLUSTRATION.

	Page
PLATE I. Photomicrograph of organic compounds isolated from soils and of characteristic derivatives of these: FIG. 1. Acid potassium saccharate; 2. Succinic acid; 3. Choline platinum chloride; 4. Trimethylamine platinum chloride; 5. Trithiobenzaldehyde; 6. Lysine picrate.....	16



SOME ORGANIC SOIL CONSTITUENTS.

INTRODUCTION.

A continuation of the investigation into the nature of the organic matter of soils, begun by the Bureau of Soils a few years ago and reported in previous bulletins,¹ has resulted in considerable additional information regarding the chemical identity of the compounds in this complex mixture.

It is the purpose of the present bulletin to report the isolation of a number of organic compounds found in soil organic matter not included in previous publications of this laboratory. The methods by which these compounds have been isolated will be described, their properties noted, and their possible origin considered.

In the reports of previous work along this line the importance of some definite chemical knowledge of the nature of the organic matter of soil, from the practical as well as the purely scientific standpoint, has been so fully discussed that it seems necessary here but to mention that all the evidence regarding the nature of this material indicates that it is of very complex composition; that nearly all classes of organic chemical compounds may be found in it; and that these compounds include some that modify the physical condition of the soil or determine the nature of the microorganisms growing therein, as well as those that may be directly harmful or beneficial to crops.

In the continuation of this work, the same general methods described in previous reports have been used, either so modified as to meet the requirements of the isolation of a definite compound sought, or, in the general search for unknown compounds, so changed as to overcome difficulties presented by the particular soil under examination.

The starting point in these methods has been generally an extraction of the soil with dilute alkali, usually a 2 per cent solution of sodium hydroxide. When the solution so obtained is acidified the so-called humous bodies are precipitated, and on filtering there is obtained an acid filtrate containing, in addition to free acid and a sodium salt, a portion of the organic matter of the soil.

¹ Bulletins Nos. 53, 74, and 83, Bu. of Soils, U. S. Dept. Agr.

All of the compounds described in this bulletin have been found in this acid filtrate and are, therefore, included in that portion of the soil organic matter soluble in cold dilute alkali and remaining in solution in part at least when this alkaline extract is acidified.

The soils examined have been for the most part samples submitted or requested because of some special problem in soil fertility. This problem may have been either a general failure of crops, or, more often, a failure of some particular crop, with the preponderance of evidence indicating unfavorable soil conditions. In some cases but a single compound was isolated from one soil and no attempt was made to study the whole of the organic matter in any one sample other than to determine the presence or absence of organic compounds such as dihydroxystearic acid, creatinine, purine bases, etc., that have been found to be rather widely distributed.

The occurrence of the compounds isolated in other soils or the distribution of these compounds in soils generally has been left for future work.

Recent improvements in the equipment of the laboratory devoted to this work have made it possible to handle much larger samples of soil than before, and the presence of compounds has in some cases been established where it would probably have been overlooked when working with smaller samples and under the limitations of the ordinary equipment.

The chief features of this equipment are a porcelain-lined kettle of 30-gallon capacity, provided with a power-driven stirrer, in which 50 pounds of soil can be treated with the solution of sodium hydroxide, and a vacuum pan in which the extract after neutralizing can be concentrated to a small volume at a comparatively low temperature. With this equipment it is possible to extract 50 pounds of soil twice, and have the final solution ready for chemical treatment concentrated to 4 or 5 gallons in three days.

ORGANIC ACIDS.

Organic acids, either as such or as salts, are present in all living vegetation. The number of such acids is large and the quantity often present is a considerable portion of the plant tissues or juices. These acids or their decomposition products become a part of the organic matter of the soil when the débris of dead vegetation is incorporated with the soil. Furthermore, organic acids are the most common products of the decay of fats, protein, and carbohydrates, and they must be formed in great variety and quantity in the course of the decomposition of plant remains in the soil.

The character of the organic acids added to the soil or formed in the soil will naturally depend on the character of the vegetation, but

probably also on the character of the microorganisms in the soil and the nature of its mineral constituents.

The tendency of nearly all organic acids in the process of decay is to change ultimately into simple gaseous products such as carbon dioxide, marsh gas, ammonia, and nitrogen; but the microflora in a soil may be such that this change is not brought about; or intermediate products may be used as food by microorganisms and built up again into new complex compounds. On the other hand, the acids in plant remains or those formed in the first stages of decay may be immediately neutralized by combination with some of the mineral constituents of the soil, resulting in insoluble or little soluble salts that may resist further change.

Soils that give an acid aqueous extract are not uncommon, a condition that can mean either the presence of a free acid, an acid salt, or a neutral salt that is hydrolyzed in water solution. Although several acids have been isolated from soils, there is as yet no conclusive evidence as to the identity of the free acids, if such exist, in acid soils. There is abundance indirect evidence that there are in soils combinations of organic acids with mineral bases, but naturally when dealing with such a complex mixture of organic and mineral matter as a soil any attempt to couple up an organic acid isolated with any single mineral constituent is purely a matter of conjecture.

OXALIC ACID, $C_2H_2O_4$.

Oxalic acid was first isolated from a soil in the examination of a sample from Hood River Valley in Oregon. This soil, a dark-colored fine sandy loam, had been for some years devoted to apple growing.

The method by which oxalic acid was isolated from this soil was as follows: An alkaline extract was acidified with acetic acid and filtered from the humus precipitate, which was small in quantity. The acid filtrate was neutralized with sodium hydroxide and concentrated in a vacuum pan to about one-fourth its volume, acidified again with acetic acid, and filtered from a further slight precipitate. Lead acetate in excess was then added to the slightly acid solution, filtered, and ammonia to strong alkalinity added to the filtrate. The precipitate thus formed was filtered off, washed, and decomposed by hydrogen sulphide. The filtrate from lead sulphide was evaporated to dryness and the dry residue extracted with ether, the ether evaporated, and the residue taken up with water and filtered. The clear, somewhat colored solution was made alkaline with ammonia and a little calcium-chloride solution added, filtered immediately from a colored flocculent precipitate that formed, and the filtrate allowed to stand in a warm place some hours, when a white, crystalline precipitate formed. This, on examination under the microscope, presented the dumb-bell shaped crystals characteristic of calcium

oxalate. These crystals were soluble in mineral acid, insoluble in acetic acid, and could be recovered again in characteristic form from acid solution by making alkaline with ammonia and heating or allowing to stand.

On evaporating a hydrochloric acid solution of this precipitate to dryness, extracting the residue with ether, crystals having the appearance, solubility, and other properties of oxalic acid were obtained on evaporation of the ether. These crystals lost water of crystallization on heating, and after drying at 100° melted at 189° . When heated with concentrated sulphuric acid, carbon dioxide and monoxide were evolved. A water solution decolorized a solution of potassium permanganate. On heating with para toluidine and washing the product with alcohol a white compound crystallizing in plates and melting at 267° was obtained. The crystalline form and melting point of this compound correspond with those of oxal-para-toluide, a compound characteristic of oxalic acid. The quantity of oxalic acid obtained from this soil by this method was small, and on the assumption that the oxalic acid might be present in the soil as calcium oxalate or as some salt little soluble and not likely to be decomposed by the short treatment with dilute alkali, recourse was had to another method to ascertain if more were present than appeared from the results obtained. The soil was leached with dilute cold hydrochloric acid, 2 to 3 per cent, and the yellow-colored extract concentrated by evaporation at a low temperature. On extracting the concentrated extract with ether a crystalline residue, having in part the appearance of oxalic acid, was obtained. This residue, when taken up with water, gave all the reactions of a solution of oxalic acid, and the quantity obtained was much greater than by the method first used. It was further found that by concentrating the acid soil extract to the point where crystallization began, allowing to cool and drying the mass on a porous plate, redissolving in water, and repeating this operation several times, a fairly pure preparation of crystallized oxalic acid could be obtained. The original extract was strongly colored with ferric chloride, and it was not possible by this method to remove the last traces of yellow tinge from the crystals of oxalic acid.

An attempt to determine the quantity of oxalic acid present in the acid extract resulted as follows: An extract made by leaching 50 grams of soil with 2 per cent hydrochloric acid until no longer colored was evaporated nearly to dryness, taken up in a little water, acidified with sulphuric acid, and heated to drive off the remaining hydrochloric acid. This solution was titrated with a standard solution of potassium permanganate and the calculation made to calcium oxalate, $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$. The mean of two closely agreeing determinations gave 0.22 per cent calcium oxalate in the air-dried soil, or assuming an

acre-foot of soil to weigh 3,500,000 pounds, 7,700 pounds, or nearly 4 tons of calcium oxalate per acre-foot.

Oxalic acid has not heretofore been found in soils, and so far its presence has been noted in but one sample, but in view of its wide distribution in plants and its common occurrence as a product of the decomposition of other organic material it seems that it or its salts might be expected to be rather common constituents of soils and of mineral deposits.

As a rock constituent, oxalates are of rare occurrence, three mineral forms only having been found and described. These are whewellite, calcium oxalate,¹ humboldtine, ferrous oxalate,² and oxammite, ammonium oxalate.³ These have been found in but a few locations, and there is no reason to suppose that the oxalic acid in the soil examined had its origin in the rocks from which the soil was formed. On the other hand, oxalates are very common in plants. As the acid potassium salt it is found in the oxalis family and a large number of other plants. It has also been found as a magnesium salt,⁴ but the most common and widely distributed form is calcium oxalate. This salt forms the raphides found in many plants, and it is also a constituent of the aleurone grains. In some of the lower plants, such as the fungi, calcium oxalate occurs in large quantity; for instance, in *Pertusaria communis* ⁵ 47 per cent of the dry weight of the plant and in *Chlorangium jusuffii* more than 65 per cent is made up of this salt.

Oxalic acid is also a very common product of the decomposition of organic plant constituents of all kinds. Protein, fats, and carbohydrates by the action of certain microorganisms or by treatment with chemical reagents yield oxalic acid as one of the products of oxidation, and it seems to be a general stage in the decomposition of organic matter just prior to the final resolution into carbon dioxide. Dihydroxystearic acid, a somewhat widely distributed soil constituent, yields oxalic acid as one of the products of oxidation.

Oxalic acid then is added to soils in the vegetable débris that goes to make up the organic matter of all soils and no doubt under certain conditions results from the decomposition of more complex organic matter in the soil. At present the data are not sufficient to warrant any general statement regarding the occurrence of oxalic acid in soils. The evidence at hand, however, indicates that its presence in the quantity found in this soil is not general and the conclusion seems warranted that oxalates, when added to the soils, rapidly disappear, or if generated in soils, the conditions under which they persist are

¹ Brooke, Phil. Mag., 16, 449 (1840).

² Review. An. Chem. Phys., 18, 207 (1821).

³ Shephard. Rural Carolinian, p. 71 (1870).

⁴ Monteverde. Botan. Centr., 43, 329 (1890).

⁵ Braconnot. An. Chem. Phys., (2) 28, 318 (1825).

For an account of the occurrence of oxalic acid in plants see Czapek, Biochemie der Pflanzen, Vol. II, pp. 417-428.

abnormal. Oxalic acid appeared in this case in the hydrochloric acid extract together with iron and calcium. There was more than enough calcium present to combine with all the oxalic acid and it would, therefore, seem probable that the oxalic acid was present in the soil as calcium oxalate.

SUCCINIC ACID, $C_4H_6O_4$.

Succinic acid was first isolated from a sandy loam from California devoted to orange culture.

A neutral solution obtained by extracting this soil in the usual way with sodium hydroxide and neutralizing with sulphuric acid, concentrating and filtering, was heated to boiling and then treated with a solution of ferric chloride to which ammonia had been added until a slight permanent precipitate was formed. The precipitate separated by filtering while hot was washed with hot water and then heated with dilute ammonia, and filtered hot. The filtrate was concentrated, barium chloride added, and a slight flocculent precipitate that formed separated by filtration. Two volumes of 95 per cent alcohol were then added and the solution filtered immediately from the flocculent precipitate and the filtrate allowed to stand, when a white crystalline precipitate was formed. This under the microscope had the somewhat characteristic appearance of barium succinate. From a water solution of this preparation by treatment with hot dilute sulphuric acid until there was no further precipitation, filtering from barium sulphate, and concentrating the filtrate, crystals were obtained having the properties of succinic acid.

The identity of this compound as succinic acid was indicated by the method of preparation which is a general one for the separation of this acid from other acids often found accompanying it, and this indication was confirmed by the properties of the crystals obtained. The compound crystallized in prisms soluble in water and alcohol, but very little in ether. It melted at 185° . A neutralized solution gave a red amorphous precipitate with ferric chloride, soluble in ammonia, and from this the barium salt could be made again in the manner already described. By heating with para-toluidine and washing the reaction product with alcohol white needles melting at 255° were obtained, the crystalline form and melting point being characteristic of succintoluide.

Succinic acid, while by no means so widely distributed in plants as oxalic acid, occurs commonly enough to be considered one of the compounds generally added to soil in vegetable debris. It has been found, for instance, in *Lactuca sativa*,¹ in Vermuth,² and *Papaver somniferum*.³ It has been found in thymus glands of calves;⁴ in the

¹ Kohuke, berg. Jahr., 25, 443.

² Zurger, Ann. Chem., 48, 122 (1843).

³ Walz, Jahr. Fortsch. Chem., 263 (1860).

⁴ Gorup, Ann. Chem., 76, 369 (1846).

urine of cows, goats, rabbits, and horses. It is also found in fossil resins, such as amber, which is its chief commercial source, in lignite, and in brown coal.¹

It is also produced by certain microorganisms in the alcoholic formation of sugar,² in the fermentation of calcium malate,³ and the putrification of flesh.⁴ By purely chemical reactions it is produced in the oxidation of fats and waxes by nitric acid.⁵

It is apparent, then, that succinic acid as an organic soil constituent might have its origin from the incorporation in the soil of resinous or lignitic material, from the incorporation of plant remains containing it, or from the decomposition of organic material. The quantity of succinic acid obtained from the soil was small and owing to contaminations that always accompanied its first separation by the method described and which could not be removed without considerable loss, no attempt was made to determine the quantity present.

Nothing is as yet known regarding its distribution in other soils. The soil from which it was isolated had received rather large applications of organic fertilizers, but whether the soil was in this way rendered abnormal in respect to the presence of succinic acid or other organic constituents is not known.

SACCHARIC ACID, $C_4H_4(OH)_4(COOH_2)_2$

Saccharic acid was first found in a soil from the Mount Vernon estate, Virginia. In the course of the investigation of this soil certain reactions indicated the presence of this acid, and this was confirmed by its isolation from the soil by the following procedure:

An alkaline extract acidified with acetic acid was filtered; neutralized, and concentrated; made slightly acid and filtered again. Lead acetate was added and the precipitate removed by filtration. Ammonia was added to the filtrate, the precipitate filtered off, washed, decomposed by hydrogen sulphide, and the filtrate from lead sulphide concentrated to a small volume. This residue was treated with several volumes of 95 per cent alcohol and filtered. The alcohol was removed by evaporation and the residue taken up with water, filtered again, neutralized with ammonia, and a solution of cadmium sulphate added. The precipitate formed was filtered off, washed, and decomposed by hydrogen sulphide, and the filtrate from cadmium sulphide concentrated. This residue was treated with 95 per cent alcohol, filtered, the alcohol evaporated, taken up by water, filtered again, and concentrated. The residue so obtained was a

¹ Reich, *Jahr. Fortsch. Chem.*, **43**, 499 (1847).

² Pasteur, *Ann. Chem.*, **105**, 264 (1858).

³ Dessaignes, *Ann. Chem.*, **70**, 102 (1849).

⁴ Salkowski, *Ber. deutsch. Chem.*, **12**, 649 (1879).

⁵ Bromeis, *Ann. Chem.*, **35**, 90 (1838); **37**, 292 (1839); Stramer, *Ann. Chem.*, **43**, 346 (1843); Ronalds, *Ann. Chem.*, **43**, 356 (1843); Radcliff, *Ann. Chem.*, **43**, 351 (1843).

thick sirup that dried in a dessicator to a horny mass and deliquesced in the air. It was soluble in water and alcohol in all proportions, but was little soluble in ether. A watery solution reduced ammoniacal silver nitrate solution, but did not reduce Fehling's solution. On neutralizing a watery solution with potassium carbonate and adding an excess of acetic acid, there formed on standing a crystalline precipitate of fine needles. This was rather difficultly soluble in cold water and had the appearance of the acid potassium salt of saccharic acid. The preparation of this salt furnishes an effective means of purifying the product obtained from the soil, and can best be applied in the method described by introducing it just preceding the precipitation with cadmium sulphate.

The identity of the compound obtained in the manner described was indicated as saccharic acid by its method of isolation, its properties, and the properties of the acid potassium salt. This identity was confirmed by comparison with pure saccharic acid prepared by the oxidation of dextrose with nitric acid and finally by analysis of the silver salt. The silver salt, prepared by neutralizing a watery solution of the acid with ammonia and precipitating with silver nitrate, gave on analysis 51.02 per cent Ag., the theoretical quantity required by the formula $\text{Ag}_2\text{C}_6\text{H}_8\text{O}_8$, being 50.94 per cent.

Saccharic acid, so far as known, does not occur in plant or animal tissues. It is formed in the laboratory by oxidation of dextrose, or of disaccharides such as sucrose or maltose, or other carbohydrates, such as starch that contain the dextrose group. It is quite possible that this oxidation may be brought about by the activity of micro-organisms, although at present oxidation by chemical reagents, usually nitric acid, is the only method known for its preparation.

Material that could yield this acid by oxidation is, of course, abundant in the vegetable material added to soils, and it seems quite reasonable to look upon saccharic acid as one of the products of the oxidation of this material in the soil. The soil from which saccharic acid was isolated was acid in reaction and abnormal in several respects, but examination of several other soils indicates that saccharic acid as a soil constituent is widely distributed and probably not a compound giving the soil from Mount Vernon its abnormal character.

ACRYLIC ACID, $\text{CH}_2\text{CH.COOH}$.

Acrylic acid was first isolated from the soil from the Mount Vernon estate, in which saccharic acid just described was found. A neutral concentrated extract prepared in the usual manner by extracting the soil with sodium hydroxide, neutralizing with acetic acid, concentrating, and filtering, was treated with an excess of lead subacetate. The precipitate was filtered off, well washed, and while still moist treated with dilute sulphuric acid slightly in excess of the

lead in the precipitate, and filtered from the lead sulphate. The dark-colored filtrate was shaken several times with ether, the ether extracts combined and shaken with a concentrated solution of sodium bisulphite. The greater portion of the coloring matter, together with aldehyde bodies, is removed from the ether by this treatment. The ether, after separation from the bisulphite solution, was allowed to evaporate spontaneously or at a low temperature on the surface of a little water, and the watery solution remaining filtered from some resinous material. The solution so obtained was slightly colored, strongly acid in reaction, and had a very sharp acetic like odor. This acid solution had the properties of acrylic acid. It decolorized a solution of potassium permanganate, and combined directly with bromine. The bromine compound crystallized in prisms soluble in ether and melted at 64° to 65° , properties characteristic of β dibrompropionic acid, a compound obtained by the direct action of bromine on acrylic acid. The acid, when neutralized, gave a crystalline precipitate with lead acetate, the crystals being needles and soluble in alcohol, properties characteristic of the lead salt of acrylic acid.

Acrylic acid has been identified in but one soil, but since its isolation and identification it has seemed likely that an acid that had previously been isolated from several soils, but not in sufficient quantity for identification, might also have been acrylic acid. These acid separations had been obtained by three separate methods and had properties, so far as ascertained, similar to those of acrylic acid. First, distillation of the soil acidified with phosphoric acid, neutralization of the slightly acid distillate with barium hydroxide, evaporation to dryness, and treatment of the residue with enough sulphuric acid to precipitate the barium and filtration from barium sulphate; second, extraction with sodium hydroxide, making acid with sulphuric acid, filtering, shaking out the filtrate with ether, and removing the ether by evaporation; third, distillation of a white crystalline crust that sometimes forms on the surface of soils in the process of drying in the field or laboratory, with sulphuric or phosphoric acid. This crust, which often collects in considerable quantities, is evidently a mixture of salts of calcium, magnesium, and other bases. Nitric acid is usually present as well as organic acids. The quantities of acids obtained by these methods have been small. In the first method there was evidently loss of acid on concentration of the neutralized distillate, either from decomposition or hydrolysis of the barium salt. The second method yielded but traces, and the distillate obtained by the third method was so complex in composition that satisfactory identification of its constituents was not possible with the quantity obtained.

Acrylic acid is not known to occur as a natural product in animal or vegetable tissues and the methods of its preparation in the labora-

tory are not such as to throw any light on the manner in which it may possibly be formed in the soil. Among these methods are the oxidation of acrolein, the oxidation of allyl alcohol, and the distillation of calcium lactate.

ORGANIC BASES.

Organic bases contain nitrogen usually linked with hydrogen and may be looked upon as ammonia with one or more hydrogen atoms substituted by other groups. These substituting groups may vary from the simple methyl or ethyl of the amines to the complex groups present in the hexone bases, or a number of substituted ammonias may be grouped together in ring form as in the pyrimidine derivatives and purine bases. Where the carboxyl group enters largely into the substitution and there is but one NH_2 group the resulting compound is mainly acid in character, but when there are two or more NH_2 groups or sometimes an NH group the compound is basic.

Those that have acid characteristics like the monamino acids can usually play the part of either an acid or a base, that is, they form salts with mineral acids, and also combine with mineral bases in the same way as ordinary acids. Members of the monamino acid group have not been found in soils except as the result of the hydrolysis of the soil organic matter with boiling acid, in which case the leucine and iso-leucine obtained were probably formed by the breaking down of more complex compounds of a protein character.¹

In a previous bulletin,² the isolation from soils of several organic compounds, basic in character, has been reported. These were histidine and arginine, diamino acids or hexone bases, cytosine a pyrimidine derivative, and xanthine and hypoxanthine, purine bases. Since then the presence of four other organic bases in soils has been established. These are lysine, a hexone base; adenine, a purine base; and choline and trimethylamine, substituted ammonia compounds.

LYSINE, $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2$.

Lysine has been isolated from but one soil, that from California in which succinic acid was found. The method of its isolation was a modification of that used in its separation from other diamino acids in the products of the hydrolysis of protein.³

An acid extract, obtained by extracting the soil with sodium hydroxide, acidifying with sulphuric acid and filtering, was treated with a solution of phosphotungstic acid in 5 per cent sulphuric acid and allowed to stand some hours. The precipitate formed was washed with dilute sulphuric acid and treated with an excess of a

¹ Robertson, Tech. Bul. No. 7. Mich. Agr. Expt. Sta. (1911).

² Bul. No. 74, Bureau of Soils.

³ Kossel & Kitcher, Z. physiol. Chem., 31, 166 (1900).

solution of barium hydroxide, filtered, and the excess of barium removed by carbon dioxide. Silver sulphate in slight excess was added to the filtrate from the barium carbonate and solid barium hydroxide added to saturation. This treatment removes histidine and arginine. The filtrate from the precipitate of these bases was acidified with sulphuric acid and treated with hydrogen sulphide, filtered, and treated again with phosphotungstic acid as in the first stage. The precipitate was washed and treated with barium hydroxide as before, excess of barium removed by carbon dioxide and the filtrate concentrated to a small volume.

The resulting sirupy solution was strongly alkaline and did not show any tendency to crystallize on standing. When taken up in alcohol and an alcoholic solution of picric acid added, a precipitate was formed having the characteristic appearance, properties, and composition of lysine picrate. This precipitate, after washing with absolute alcohol and dissolving in hot water, separates on cooling in rather large prisms, or if only a small quantity is treated, in fern-like aggregates. Lysine picrate is difficultly soluble in cold water, crystallizes in characteristic form, and has the composition, carbon, 38.40 per cent; hydrogen, 4.53 per cent; nitrogen, 18.67 per cent.

Elementary analysis of the preparation from soil gave carbon, 38.28 per cent; hydrogen, 4.65 per cent; nitrogen, 18.70 per cent. Lysine picrate is the most characteristic salt of lysine and its preparation and analysis can be depended upon for identification. From the picrate, lysine hydrochloride was prepared. This salt, $C_6H_{14}N_2O_2 \cdot 2HCl$, is crystalline; very little soluble in alcohol, but readily soluble in water, and crystallizes from watery solution on concentration. It melts at 192° to 193° . The compound prepared from the soil showed the same properties and melting point.

Structurally, lysine is a diamino caproic acid and is represented by the formula $CH_2NH_2(CH_2)_3CH.NH_2.COOH$. It is a product of the decomposition of protein and results from such decomposition in the case of all but a few vegetable proteins and some protamines. It is not known to occur as such in any vegetable or animal tissue and is usually prepared by the acid hydrolysis of protein material such as casein or gelatine.

As a constituent of soil organic matter, it can be regarded as one of the products of the decomposition of protein material in the soil. The quantity obtained from the soil in which it was found was small. Several other soils have been examined for lysine but its presence could not be shown and it does not seem to be a common constituent of the organic matter of soils.

ADENINE, $C_5H_5N_5$.

The isolation of two of the commonly occurring purine bases, xanthine and hypoxanthine, from soils has already been recorded ¹

These have been found to be somewhat widely distributed in soils, but two others that are also common decomposition products of nucleo-proteids, guanine,² and adenine seem not to be so common constituents of the organic matter of soils.

Adenine was first isolated from a soil from near Redding, Conn. The method used in the isolation of adenine was in the first stages the same as used in the preparation of xanthine and hypoxanthine. An alkaline extract, to which Fehling's solution had been added, was heated to boiling and a little dextrose added. The precipitate formed was removed by filtration, washed, decomposed by hydrogen sulphide, and the filtrate from copper sulphide concentrated to a small volume. Tests of a small portion of this solution by evaporation to dryness with nitric acid, addition of alkali, and heating developed no red or pink color, showing the absence of xanthine or guanine in the solution. Another portion gave a fine red color with ferric chloride, which color was unchanged by heating, a reaction characteristic of adenine. To the main portion of the solution picric acid was then added, when there was an immediate formation of bunches of fine needles. These crystals were difficultly soluble in cold water and on recrystallization from hot water melted at 180° , which is the melting point of adenine picrate.

The compound obtained by this method was identified as a purine base by its method of isolation. The absence of xanthine and guanine being established, only hypoxanthine and adenine of the common purine bases were left to be considered. The precipitate produced by picric acid fixed the identity as adenine, since hypoxanthine picrate is quite soluble. The identity of the picrate as adenine picrate was further established by its crystalline form, solubility, and melting point.

From a portion of the original adenine solution obtained as described above several other salts were prepared. Adenine bichromate, crystallizing in six-sided plates difficultly soluble in cold water, separated on allowing a mixture of adenine and chromic acid solutions to stand several hours. On evaporating a solution of adenine with hydrochloric acid and gold chloride, the characteristic double salt separated in long orange-colored prisms. Adenine hydrochloride was obtained by evaporating the adenine solution with hydrochloric acid. It crystallized in prisms easily soluble in water. The original adenine solution, when evaporated, yielded a crystalline residue of

¹ Bul. Nos. 74 and 80, Bureau of Soils, U. S. Dept. of Agr.

² Guanine has been found in heated soil by Mr. E. C. Lathrop, of this laboratory, and will be reported later.



FIG. 1. ACID POTASSIUM SACCHARATE.

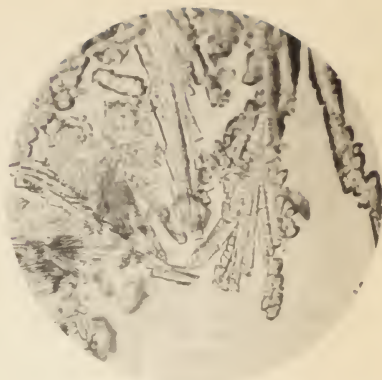


FIG. 2. SUCCINIC ACID.

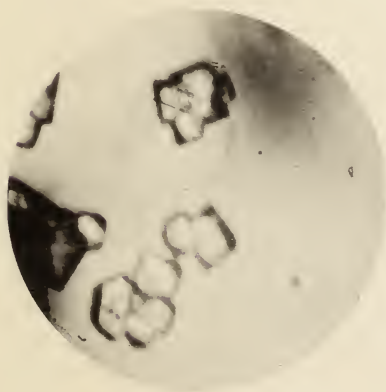


FIG. 3. CHOLINE PLATINUM CHLORIDE.

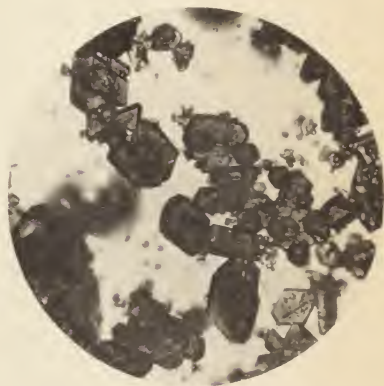


FIG. 4. TRIMETHYLAMINE PLATINUM CHLORIDE.

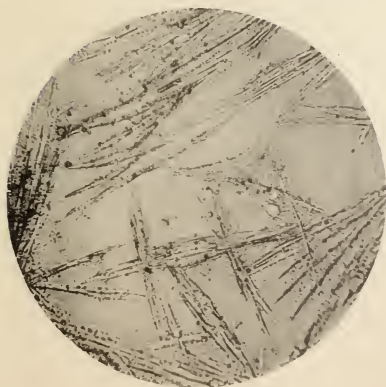


FIG. 5. TRITHIOBENZALDEHYDE.

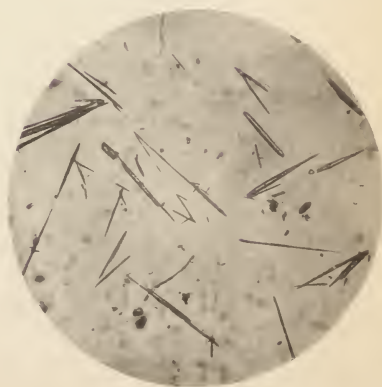


FIG. 6. LYSINE PICRATE.

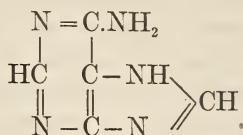
PHOTOMICROGRAPH OF ORGANIC COMPOUNDS ISOLATED FROM SOILS AND OF
CHARACTERISTIC DERIVATIVES OF THESE.



microscopic needles, soluble rather easily in water but little or not at all in alcohol or ether.

In the application of this method to soils generally the solution finally obtained would contain creatine or creatinine if present in the soil in addition to the purine bases. In this case the solution apparently was one of nearly pure adenine, no other purine bases being present, and but traces of creatinine.

Adenine structurally is 6-amino purine and is represented by the formula,



It was first prepared from the pancreatic glands and is found in all animal tissues or fluids rich in nucleated cells. It has been found in products of the autodigestion of yeast and in several plants. It is most conveniently prepared from an extract of tea leaves.

The soil from which adenine was obtained was rather abnormal in several respects. It was sandy loam, a woodland soil, uncultivated and bearing sparse vegetation. It had been found to contain a relatively large quantity of dihydroxystearic acid. Since its isolation from this soil adenine has been obtained from other soils, but apparently is not so common a constituent as xanthine or hypoxanthine. Adenine, like the other purine bases, occurs as a decomposition product of nucleic acid, and its presence in soils may be due to the addition as such in plant or animal matter or to the decomposition of antecedent compounds in the soil.

CHOLINE, $\text{C}_5\text{H}_{15}\text{NO}_2$.

Choline, an organic basic compound represented by the structural formula $\text{C}_2\text{H}_4\text{OH} . \text{N}(\text{CH}_3)_3\text{OH}$, or hydroxy-ethyl-trimethyl ammonium hydroxide, has been isolated from a sample of Volusia silt loam from New York. The method of isolation depended on the precipitation of the compound with mercuric chloride and was carried out in the following manner: An alkaline extract of the soil was acidified with acetic acid, filtered, neutralized with sodium hydroxide, and filtered again. To the neutral filtrate lead acetate was added as long as a precipitate was formed, filtered, and excess of lead removed from the filtrate by hydrogen sulphide. The filtrate was concentrated to a similar volume, treated with several volumes of alcohol, a little hydrochloric acid added, and the material insoluble removed by filtration. To this filtrate an alcoholic solution of mercuric chloride was added and the whole allowed to stand several days. The precipitate which formed was separated, dissolved in water, the watery solution con-

centrated, and the crystals that separated dissolved again in water and treated with hydrogen sulphide. The filtrate from mercuric sulphide on concentration yielded crystals having the properties of choline hydrochloride.

A watery solution gave precipitates with phosphotungstic and phosphomolybdic acids, with mercuric and gold chlorides and with iodine in potassium iodide solution. The precipitate with gold chloride was soluble in hot water and alcohol. It crystallized from water solutions in small prisms and needles that melted at 239° to 240° , and on analysis was shown to contain 44.40 per cent Au., the theoretical gold content for $C_5H_{14}NO \cdot Cl \cdot AuCl_3$, being 44.48 per cent. On addition of platinum chloride and concentration orange-red six-sided plates were formed, easily soluble in water but insoluble in alcohol. These melted at 230° to 233° , the melting point of choline platinum chloride being given by different authorities at 213° to 240° .

On treating this solution of choline hydrochloride with silver oxide, filtering from silver chloride, and concentrating, a sirupy liquid was obtained, strongly alkaline in reaction and having all the properties of choline. From this the hydrochloride, mercuric, gold, and platinum double salts could be obtained again in crystalline form.

The quantity of choline isolated from this soil was small and would probably have escaped detection in a general search for organic compounds. The special search for choline has not been applied to other soils and no statement can be made regarding its distribution in soils.

Choline is widely distributed in both animal and vegetable tissues and is also one of the products in the first stages of the decomposition of more complex compounds in these tissues. The presence of choline in soils may then be due either to its direct addition in organic débris or to the decomposition of more complex compounds in the soil.

TRIMETHYLAMINE, C_3H_9N .

The fishy ammoniacal odor characteristic of the monamines is sometimes observed when the soil is treated with cold dilute alkali, as in the method of extraction of the soil organic matter used throughout these researches. In the case of one soil, a salt marsh soil from Georgia, this odor was so pronounced and persistent that it was thought it might be possible to determine the identity of the amine present. For this purpose the soil was treated with sufficient water and magnesium oxide to make a thin mud strongly alkaline in reaction. This was distilled under reduced pressure, the distillate made acid with hydrochloric acid, and evaporated to dryness. In this way a quantity of crystalline material soluble in water was obtained, which, when made alkaline, had the odor of amines and

ammonia, the latter predominating so strongly that it was evident that ammonium chloride made up a large proportion of the mixture. Efforts to separate the amine chlorides from ammonium chloride by treatment with absolute alcohol, in which the latter is stated to be insoluble, resulted in but a partial separation. The alcohol soluble portion contained, after several treatments, considerable ammonia. After this partial separation of ammonium chloride, the alcohol soluble portion, after evaporation of the alcohol, was taken up in water and this solution gave reactions characteristic of trimethylamine. When acidified with hydrochloric acid and potassium ferrocyanide added, a white crystalline precipitate quite insoluble in water was formed. The formation of this precipitate under these conditions indicated the presence of a tertiary monamine. On treatment of this acid ferrocyanide with a solution of copper sulphate, filtering, and removing sulphuric acid and excess of copper sulphate from the filtrate by the addition of a solution of barium hydroxide there was obtained a solution having a strong amine odor. On addition of platinum chloride to a solution of the mixed chlorides there was formed a precipitate in which crystals having the form of both ammonium chloroplatinate and trimethylamine chloroplatinate were observed, but it was not possible with the material available to separate the two completely enough to make further tests of the identity of the latter. Other attempts to separate completely the relatively larger amount of ammonium chloride obtained by the method used and to prepare other characteristic salts of trimethylamine or other amines failed. The evidence of the presence of trimethylamine in this soil rests first on the amine odor of the distillate obtained by distilling the soil with magnesium oxide, and second, on the precipitate of an acid ferrocyanide obtained when potassium ferrocyanide and hydrochloric acid were added to the neutralized and concentrated distillate. Third, that the amine compound could be recovered from this ferrocyanide precipitate, and fourth, on the formation of the chloroplatinate.

Trimethylamine occurs as such in both animal and vegetable products. It has been found in *Chenopodium vulvaria*,¹ *Cratogeomys oxyacantha*,² and other plants, and is one of the products of the decomposition of choline just described. As a soil constituent it therefore might be the result of direct addition in plant debris or a decomposition product of some such compound as choline.

ALDEHYDES.

Compounds of an aldehyde nature seem to be a rather common constituent of soil organic matter, but up to the present the identity of one only has been established.

¹ Dessaignes, Compt. Rend., 33, 358 (1851).

² Wicke, Ann. Chem., 91, 121 (1854).

SALICYLIC ALDEHYDE, $C_6H_4.OH.CO.H$.

Salicylic aldehyde was isolated from a sample of a soil from the Mount Vernon estate, Virginia, the same soil in which saccharic acid previously described was found. It was obtained in the following manner:

The usual sodium hydroxide extract was acidified with sulphuric acid and filtered. The acid filtrate was shaken out with several portions of ether, the ether extracts combined and shaken with a concentrated aqueous solution of sodium bisulphite. The bisulphite solution was separated from the ether, strongly acidified with sulphuric acid and air blown through it to remove sulphur dioxide. This solution was then shaken with several portions of fresh ether, the ether extracts combined, and the ether removed by evaporation over a small volume of water. The solution remaining was filtered from a small quantity of resinous insoluble material, and as thus obtained was a slightly colored solution, had an aromatic odor, and the properties of a solution of salicylic aldehyde. On slow evaporation of the water there was left a yellow oil somewhat difficultly soluble in water, but very soluble in alcohol or ether. The water solution developed a pink color in fuchsine aldehyde reagent almost immediately and gave an intense violet color with ferric chloride. When treated with phenylhydrazine, a precipitate was formed, which, on recrystallization from alcohol, was in the form of yellow leaflets that melted at 143° , the characteristic form and melting point of the phenylhydrazone of salicylic aldehyde. The separation of this compound from ether solution by an aqueous solution of sodium bisulphite and the color produced with fuchsine reagent fix it as an aldehyde. The general properties of the compound and the formation of the hydrazone melting at 143° are sufficient to identify it as salicylic aldehyde.

Salicylic aldehyde occurs in several plants, in several species of *Spirea*,¹ and in *Crepis foetida*.²

It is also formed by the oxidation of salicin, a glucoside that occurs in many species of *Salix* and *Spirea*. Its occurrence in soil then may be due to the addition of the aldehyde itself, or bodies yielding it in vegetable remains.

UNIDENTIFIED ALDEHYDES.

Salicylic aldehyde has been isolated from but one soil, but many other soils when examined according to the method by which salicylic aldehyde was obtained yield a compound or mixture of compounds having aldehyde properties, but differing from salicylic aldehyde.

¹ Ettling, Ann. Chem., 35, 247 (1838); Wicke, ibid., 83, 175 (1855).

² Wicke, Ann. Chem., 91, 374 (1856).

Usually when the aqueous solution of this material is evaporated there is a solid residue, somewhat soluble in water, and easily soluble in alcohol and ether. The water solution gives a violet color with ferric chloride and usually gives no color with fuschine reagent unless previously boiled for a few minutes with strong hydrochloric acid. No crystals have been observed in the solid residue and it has not been possible to prepare a crystalline phenylhydrazone or other derivative. The appearance and properties of this material suggest polymerized aldehydes but the quantity obtained has been too small to establish their identity.

CARBOHYDRATES.

Carbohydrates, represented by starch, sugars, and cellulose, make up a large portion of plant tissues and material in solution in the juices of plants. The ready susceptibility of the sugars and starches to the action of enzymes and microorganisms renders it probable that these plant constituents rapidly disappear from the tissues when a plant dies and are less likely to be incorporated with the soil than other organic compounds of plants. Evidence of the occurrence of carbohydrates in soils has heretofore been confined to the demonstration of the presence of pentosans or pentose-yielding bodies,¹ and, as pointed out, the pentose sugar might have been derived from complex ligno-cellulose, from nucleic acids, or from pentose-yielding glucosides, any or all of which might be fairly resistant to decomposition.

It seems evident from examination of a large number of soils that pentose-yielding material is a constant constituent of the organic matter of soils.

Two compounds isolated from soils, mannite and rhamnose, are classed in this carbohydrate group, although the term "sugars" perhaps would be the more proper designation. Rhamnose is present in the soil as a glucoside, from which it is set free in heating with a mineral acid. Mannite, although a hexatomic alcohol, is so closely related to the sugars that it is included in this section.

MANNITE, $C_6H_{14}O_6$.

Mannite was found in the soil from the Mount Vernon estate already mentioned in connection with the isolation of saccharic and acrylic acids and salicylic aldehyde. The sodium hydroxide extract was acidified with sulphuric acid, filtered, neutralized, and concentrated. To this solution, slightly acidified with acetic acid, lead acetate was added until there was no further precipitate of lead sulphate, filtered at once, and ammonia and a little more lead acetate

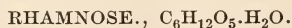
¹ Bul. No. 74, Bureau of Soils, U. S. Dept. of Agr.

added to the filtrate and the whole allowed to stand 24 hours. It was found that the first treatment with sulphuric acid and filtration removed material that could not be removed by acetic acid, and that this material, unless removed, interfered greatly with the separation and purification of the final product. The precipitate formed by ammoniacal lead acetate was collected, washed, decomposed by hydrogen sulphide, and the filtrate from lead sulphide concentrated to a small volume. On cooling, crystals in the form of needles and prisms formed. These were removed from the mother liquor, dried on a porous plate, and recrystallized several times from water. The crystals thus obtained were soluble in water and alcohol but insoluble in ether. They melted at 166° , agreeing with the melting point of mannite. The water solution was optically inactive, but on the addition of borax became markedly dextro rotatory. The solution did not reduce Fehling's solution or ammoniacal silver, but did reduce a solution of silver acetate.

By treatment of the compound with concentrated hydrochloric acid and benzaldehyde, crystals were formed, difficultly soluble in acid. The compound crystallized in needles and melted at 191° , characteristic properties of mannite-tribenzacetol formed from mannite in this way.¹ The identity of the compound isolated from the soil in the way described is indicated as mannite by its method of preparation, its crystalline form, solubility, and melting point, and this identity is confirmed by the optical properties of the solution and the formation of the compound having the properties of mannite-tribenzacetol when subjected to treatment known to give this compound.

Mannite occurs as a natural product in a large number of plants—in celery, *Apium graveolus*,² in *Canella alba*,³ in *Aconitum napellus*,⁴ and in *Syringa vulgaris*.⁵ It is found in large quantities in several species of fungi; for instance, *Agaricus integer* may contain as much as 20 per cent of its dry weight of mannite.⁶

It is also found as one of the products of certain fermentations of sugar, and by the reduction of dextrose, levulose, or mannose. Its occurrence in soils may be as with many other organic compounds, the result of either a direct addition in plant remains or one of the products of decomposition of other compounds in the soil. The quantity obtained from the soil in which it was found was relatively large, amounting to at least 500 pounds per acre-foot. It has not yet been encountered in any other soil.



A glucoside, yielding rhamnose on hydrolysis with mineral acids, was isolated from a soil from New York, the same Volusia silt loam

¹ Fischer, Deutsch Chem., 27, 1524 (1894).

² Payen, Ann. Chem., 12, 60 (1835).

³ Meyer, Reiche. Ann. Chem., 47, 234 (1843).

⁴ Smith, Jahr. Fortsch. Chem., 535 (1850).

⁵ Ludwig, Jahr. Fortsch. Chem., 503 (1857).

⁶ Thorner, Ber. deutsch Chem., 12, 1635 (1879).

from which choline, already described, was obtained. The method by which the glucoside was obtained was as follows:

The usual sodium hydroxide extract was acidified with acetic acid and filtered. The acid filtrate was neutralized, filtered, and made slightly acid again with acetic acid. To this acid solution lead acetate was added until a precipitate no longer formed and the precipitate removed, washed, and decomposed by hydrogen sulphide. The filtrate from lead sulphide on evaporation to dryness yielded a dark-colored amorphous mass, soluble in water, but yielding no crystals, and having few distinctive properties other than yielding a precipitate with a number of metallic salts. A water solution of this material gave, after short boiling with a mineral acid, a solution that reduced Fehling's solution, indicating that the dark-colored amorphous body was a glucoside or contained a glucoside. On treating a sufficient quantity of this material with boiling dilute sulphuric acid for a short time, filtering from the dark-colored insoluble material formed, neutralizing the filtrate with barium carbonate and evaporating the filtrate to a sirup, crystals were obtained having the properties of rhamnose, a methyl pentose sugar.

These crystals, resembling very much those of cane sugar, were soluble in water and alcohol, were dextro rotatory in water solution, and reduced Fehling's solution and ammoniacal silver nitrate. On distilling with dilute mineral acid, the distillate gave the furfural test with aniline acetate, and on adding phloroglucine and collecting the precipitate it was found to be soluble in alcohol, showing it to be methyl furfural phloroglucide and that the sugar was a methyl pentose.

The crystals of the sugar when purified by several recrystallizations melted at 92–93°, the melting point of rhamnose. By treating the sugar with phenylhydrazine hydrochloride and sodium acetate for several hours on the water bath, bunches of yellow needles were obtained, difficultly soluble in warm water and melting at 180°, properties characteristic of rhamnose phenylhydrazone.¹

These properties fix the identity of the sugar as rhamnose (iso dulcitate), a methyl pentose represented by the formula:



Rhamnose is not known to occur as such in plants, but it is a splitting product of a number of glucosides of widespread occurrence in vegetation. As such it is part of the glucosides, quercitrin,² found in several species of *Quercus*, xanthorhamnin,³ in Persian berries; rutin, found in rue and buckwheat;⁴ hesperidin,⁵ in unripe oranges, and others.

¹ Fischer, *Tafel, Ber. deutsch Chem.*, 20, 1091 (1887).

⁴ Forster, *Ber. deutsch, Chem.*, 15, 215 (1882).

² Halaswetz, *Pfandler. Ann. Chem.*, 127, 362 (1863).

⁵ Will, *Ber. deutsch, Chem.*, 20, 1187 (1887).

³ Lieberman, *Hormann, Ann. Chem.*, 196, 323 (1879).

It is evident that the presence of a rhamnose yielding glucoside in soil can be easily explained by the probable addition of such a glucoside in vegetable remains. The glucosides that give rhamnose on hydrolysis yield at the same time another body, in most cases, of unknown constitution and often colored. The material formed on heating the soil glucoside with acid was dark colored, little soluble in water, but soluble in alcohol. This body could not be obtained crystalline, and nothing as yet is known regarding its nature or constitution.

The quantity of the rhamnose yielding glucoside obtained from the soil was comparatively large and made up a large proportion of the colored material precipitated by lead acetate from slightly acid solution. Whether this glucoside or similar ones are of common occurrence in soils has not been determined.

ORGANIC SULPHUR COMPOUNDS.

Sulphur is present in organic combination in all plants and animals. It is a constituent part of the protein molecule, no matter of what origin, with the exception of protamines, peptones, and mycoproteid. In plants sulphur is also found in organic form in a number of compounds present in essential oils.

In protein the sulphur is a part of the complex molecule and on hydrolysis it appears as cystein, a thioamine glyceric acid disulphide. Further decomposition may result in the formation of thiolactic acids, mercaptans, ethyl sulphide, or hydrogen sulphide. In essential oils, sulphur may be present in a variety of forms, chiefly organic sulphides or thiocarbamides.

With the knowledge that organic sulphur compounds are added to the soil with every addition of vegetable or animal organic material, it might be expected that some of these compounds would persist in the soil and form a part of the soil organic matter. Many soils give reactions indicative of the presence of organic sulphur; for instance, an alkaline extract to which lead acetate has been added may give, on boiling, a precipitate of lead sulphide, or on boiling the soil with a solution of sodium hydroxide in which a silver coin or piece of silver foil has been placed silver sulphide is formed and coats the coin. Examination of such soils for the presence of cystein, cystine, and thiolactic acids has so far been without any conclusive evidence of the presence of any of these compounds.

But one organic sulphur compound has as yet been isolated from soils. This is trithiobenzaldehyde. Soils containing free sulphur or from which free sulphur may be obtained by steam distillation are rather common. As it seems likely that this free sulphur may be formed from organic sulphur compounds through the activity of micro-organisms in the soil, some discussion of it is included under this heading.

SULPHUR.

Free sulphur has been found in a number of soils; in a few cases in a rather large quantity. The method by which free sulphur has been usually obtained is steam distillation. The soil mixed with several volumes of water, placed in a flask or other container, is subjected to a current of steam from a convenient source, the soil container at the same time being kept near the boiling point to prevent undue condensation of steam. The distillate on shaking with chloroform and evaporation of the solvent leaves a residue containing sulphur if any is driven over by the steam. If much sulphur is present it will be apparent, floating on or in the distillate, or may collect in the condenser. All rubber tubing connections and rubber stoppers must of course be avoided in carrying out this process and the freedom of the steam from oil or other impurities be assured.

In one case 0.2 gram of sulphur was obtained from 2,000 grams of soil by this method, in which case the greater part of the sulphur collected in the condenser. In the case of this soil, sulphur could be obtained also by direct extraction with chloroform, but not in so pure a form as by distillation. The soil was, however, exceptional, and the average soil that yields sulphur by this treatment gives but traces sufficient for identification when working with quantities of soil up to 2,000 grams. The source of the sulphur in such cases can be at present but a matter of conjecture.

TRITHIOBENZALDEHYDE, $(C_6H_5CSH)_3$.

Trithiobenzaldehyde was first found in the soil from California, in which succinic acid and lysine, previously described, were found. A sodium hydroxide extract of the soil was acidified with acetic acid and filtered. The acid filtrate was shaken out with several portions of ether, the ether extracts combined and allowed to evaporate at a low temperature on the surface of a little water. There was left an oily residue floating on the surface that in a short time became a mass of rather long interlacing needles, mixed with oily noncrystalline material. This mass was removed from the water by filtration and washed with small portions of cold absolute alcohol. The alcohol removed the greater portion of the oily material and but little of the crystals. These were dissolved again in ether and the process repeated until they were obtained free from color and from adhering impurities.

Thus obtained, this compound was in the form of needles that melted at 225° to 226° , agreeing with the melting point of trithiobenzaldehyde. They were difficultly soluble in alcohol and insoluble or very slightly soluble in water. They were readily soluble in hot acetic acid, separating from this solution on cooling. On heating the

crystals with finely divided copper, copper sulphide was formed, and a crystalline sublimate collected on the cool portion of the tube. The copper sulphide gave off hydrogen sulphide readily in treating with dilute hydrochloric acid, and the crystalline deposit melted at 123° , and had the other properties of stilbene or toluylene, $C_6H_5CH=C_{10}C_6H_5$, known to be formed when trithiobenzaldehyde is heated with copper.

The appearance, melting point, and solubility of the compound obtained from the soil indicated its identity as trithiobenzaldehyde, and the reaction and products obtained on heating with copper confirmed this identification. It was fully confirmed by comparison with the pure product obtained by treating an alcoholic solution of benzaldehyde with hydrogen sulphide and adding a solution of iodine in benzol to the product thus obtained. Trithiobenzaldehyde, as a laboratory product, occurs in three forms—a polymerized form, melting at 83° to 85° , obtained by the action of hydrogen sulphide on an alcoholic solution of benzaldehyde, as mentioned above. This, by the action of iodine, is changed to the β form, melting at 225° to 226° , the form obtained from the soil. Another form is obtained together with the β form when a hydrochloric alcohol solution of benzaldehyde is treated with hydrogen sulphide. This, known as the α form, melts at 166° to 167° , and is like the polymerized form, readily changed to the β form by the action of iodine.

Trithiobenzaldehyde is not known to occur as a natural product in plants or animals, and it is difficult to see any connection between it and any of the organic sulphur compounds known to occur in animal or vegetable tissues. Amygdaline, a glucoside occurring in peach kernels, the leaves of the cherry, laurel, and a number of other plants, yields benzaldehyde, as one of the splitting products. It is possible perhaps that through the action of microorganisms in the soil hydrogen sulphide is produced from organic sulphur compounds of plant or animal origin, and this acting on the benzaldehyde forms the trithiobenzaldehyde.

Trithiobenzaldehyde was also found in another soil, the salt marsh soil from Georgia, in which trimethylamine was found. The quantity obtained from the soil from California was relatively large, several hundred pounds per acre-foot; that from the salt-marsh soil was much less.

A determination of the total sulphur in the California soil by fusion with sodium carbonate and a little nitrate gave 0.59 per cent S.¹

An attempt to determine the sulphur present as trithiobenzaldehyde gave 0.18 per cent S., equivalent to 0.68 per cent trithiobenzaldehyde. The method used in this case was repeated extraction of the soil with boiling glacial acetic acid, evaporation of the extract

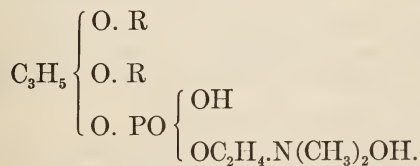
¹ Determination by Mr. W. O. Robinson, of this bureau.

to dryness, and extraction of the residue with ether. The residue left on evaporation of the ether was then subjected to fusion with sodium carbonate and nitrate and the usual procedure followed, the sulphur found as barium sulphate being calculated to trithiobenzaldehyde.

It was found possible by working with portions of soil of 200 grams to obtain crystalline preparations of trithiobenzaldehyde from the California soil by extraction with hot acetic acid, as in the method just described, and purifying the ether extract as in the method first used. The method by which this compound was first isolated, by shaking out with ether from an acid solution, is in all particulars up to the purification of the compound, the same as that used in isolating dihydroxystearic acid. As nearly all soils examined in this laboratory are subjected to this procedure for dihydroxystearic acid, trithiobenzaldehyde would be encountered and recognized if present. In the two soils in which it was found there was no evidence of the presence of dihydroxystearic acid, and while there have been a few cases when there was evidence of traces of trithiobenzaldehyde with dihydroxystearic acid, they have been few. This sulphur compound seems to be of rather infrequent occurrence.

ORGANIC PHOSPHORUS COMPOUNDS.

Phosphorus in organic combination is, like sulphur, constantly present in the tissues and fluids of plants and animals. The forms in which phosphorus occurs in organic combination may be classed under three heads: First, in combination with or as a part of a protein molecule. Nucleo-proteids represent the former and nucleo-albumins or phospho-globulins the latter. Both in breaking down yield phosphoric acid as one of the final products. Second, phosphatides; lecithins are typical of this group. The lecithins are complex compounds analogous to the glycerides and may be represented by the formula:



Where R is a fatty acid radical and the third hydroxyl is replaced by a molecule of the choline ester of glycerophosphoric acid. The different forms of lecithins are determined by the nature of the fatty acid radical present. They split off glycerophosphoric acid readily and yield finally under proper conditions free phosphoric acid. Third. Phytin, the calcium magnesium salt of an acid claimed by

Pasternak¹ to be anhydro dimethylene diphosphoric acid, and by Neuberg,² Winterstein,³ and others, to be inosite phosphoric acid. Phytin is of very widespread occurrence in plants, and is the form in which the greater portion of the organic phosphorus is present in vegetation.

It is evident, then, that organic phosphorus compounds are added to soils in every addition of animal or vegetable material, and it is reasonable to expect that some of them would persist for a time at least and make up a portion of the organic matter of the soil. Either with this idea in mind or because of the phosphorus content of fractions of soil organic matter, the question of the presence of organic phosphorus compounds in the soil has had a prominent place in all investigations of this material.

Mulder early observed that the organic matter of soils was not readily freed from phosphorus.⁴ From Mulder's time to the present discussion interspersed with some investigation has kept this question rather prominently before agricultural chemists, and very diverse views have been put forward, backed by more or less experimental evidence. Some investigators contend that the phosphoric acid that accompanied the soil organic matter through various operations was simply held by absorption, while others maintained that it was part of an organic molecule.

Schmoeger was probably the first to recognize the possibility of the occurrence in soil of organic phosphorus compounds present in plants and animals⁵ and from the increased amount of water soluble phosphoric acid present after heating soil to 150° to 160°, he concluded that nuclein or some related body was present in soils. Later Aso⁶ from similar evidence, drew the conclusion that nuclein and lecithins were present in soils.

Later investigations and discussions have ranged around the problem of methods of distinguishing between organic and inorganic phosphorus, or quantitative methods of determining the two forms both in soils and organic material generally. In soil investigations along this line there has been no isolation of any organic phosphorus compound nor any conclusive evidence of the presence of any definite organic phosphorus compound in soils.

The isolation of nucleic acid from soils in this laboratory has already been announced⁷ and is at present the only representative of this class isolated and identified. The isolation of choline, a decomposition product of lecithin, has already been described and some evidence has

¹ Compt. Rend., **137**, 439 (1903).

² Biochem. Z., **9**, 557 (1908).

³ Z. physiol. Chem., **58**, 118 (1908).

⁴ J. prakt. Chem., **32**, 326 (1844).

⁵ Ber. deutsch Chem., **26**, 386 (1893); Biedermann's Centr. Agr. Chem., **26**, 519 (1897).

⁶ Biedermann's Centr. Agr. Chem., **34**, 3 (1904).

⁷ Shorey, Science, **35**, 390 (1912); Biochem. Bul. I, 104 (1911).

been obtained of the presence of inosite, a decomposition product of phytin, but as yet this compound has not been isolated.

NUCLEIC ACID.

Nucleic acid has been isolated from a number of soils, including the Volusia silt loam and the soil from California already mentioned in connection with other compounds.

The method used in this isolation was as follows: The soil was extracted as usual with sodium hydroxide solution. Best results were obtained by using a weaker solution than ordinary, about 1 per cent, and allowing it to act for a short time only. This extract was slightly acidified with hydrochloric acid and filtered. To the acid filtrate a little sodium acetate was added and then three or four volumes of 95 per cent alcohol and the whole allowed to stand some hours. The precipitate was removed by filtration, dissolved again in weak alkali and reprecipitated in the same manner as before. The precipitate, filtered off and washed with 95 per cent alcohol, when dried was an amorphous mass, more or less colored according to the nature of the soil, and contained sometimes rather a large quantity of mineral matter that it was impossible to remove without decomposition of the organic portion. This material had the properties of nucleic acid and on hydrolysis with mineral acid gave the decomposition products characteristic of this group of phosphorus compounds.

On boiling with dilute hydrochloric or dilute sulphuric acid, phosphoric acid was found in solution and could be precipitated under proper conditions with ammonium molybdate and then determined quantitatively by precipitation with magnesia mixture. The same solution contained also pentose sugars, the solutions giving at once the pentose reactions with phloroglucine and orcin, reduced Fehling's solution, were optically active, and gave an osazone with phenylhydrazine, and on continued boiling with dilute acid furfural was given off. The resulting solution in some cases gave tests indicating the presence of levulinic acid, a precipitate of iodoform on adding sodium hydroxide and iodine potassium iodide, and a red color with sodium nitro-prusside and sodium hydroxide. When the original material was heated with stronger acid, 25 per cent sulphuric, for a short time, the solution neutralized and then treated with Fehling's solution as in the method for the separation of purine bases, hypoxanthine and in one case adenine, were obtained in the final filtrate from copper sulphide. On heating the material with 25 per cent sulphuric acid under pressure, 1 to 2 atmospheres, for a short time, the solution neutralized and filtered, cytosine was found in the filtrate. This was separated by precipitation with silver nitrate in solution made slightly alkaline with ammonia.

These reactions and the properties of the material itself and the method of preparation are sufficient to establish its identity as nucleic acid.

Nucleic acids are complex compounds for the most part of unknown constitution. They are found in all nucleated cells of both plants and animals and in such places are a part of a still more complex molecule of nucleoprotein.

Nucleoprotein when split up by chemical treatment or in the changes that take place in the living organism yields first a protein and a body known as nuclein, and the latter breaks up still further into another protein and nucleic acid. The nucleic acids, of which there are apparently a number differing in composition and constitution according to their source, are distinguished by certain general properties and the products they yield on hydrolysis. As ordinarily obtained they are amorphous compounds, soluble in alkalis and rather resistant to change in alkaline solution. They are precipitated from alkaline solution by mineral acids in the presence of a large volume of alcohol. Some of them are precipitated by this treatment only when sodium acetate is present also.

On hydrolysis they always yield phosphoric acid, a pentose sugar, purine bases, and pyrimidine derivatives. The nucleic acids prepared from soils, while differing considerably in composition according to the nature of the soil, were constant in yielding phosphoric acid, pentoses, one or more purine bases, and a pyrimidine derivative. Among the purine bases xanthine and guanine have not yet been detected in the hydrolysis products and but one pyrimidine derivative cytosin has been obtained. The widespread distribution of nucleic acids in plants and animals and their resistance to decomposition under certain conditions indicates that their occurrence in soils is due either to their persistence after addition to the soil in plant or animal remains, or to their building up by microorganisms in the soil from other less complex material.

So far as observed nucleic acids seem to be constant constituents of the organic matter of soils, and in the absence of a method of determining them quantitatively in such a complex body as the soil it can only be stated that the evidence is that in many cases they form a very appreciable portion of the organic matter of soils.

GENERAL METHODS.

In the foregoing pages methods have been described by which 14 organic compounds have been isolated from soils. These, added to the number reported in previous bulletins, make the total number of definite organic compounds isolated from soil 35. Among those described in this bulletin are members of four classes of organic compounds not represented in previous reports, viz, aldehydes, glucosides,

organic sulphur, and organic phosphorus compounds. Some of the compounds have been found as the result of a direct search for them—for instance, choline and lysine; others have been found accidentally in the carrying out of methods for compounds before isolated—for instance, trithiobenzaldehyde; and still others have resulted from modifications of methods made often for no other reason than that such modifications often bring unexpected results. This results in considerable complexity of methods, and as the work progresses the complexity promises to increase and makes desirable some general discussion of the methods that have been used.

ALKALINE EXTRACTION.

Investigation has shown the presence in soils of a number of organic compounds that are quite soluble in water. These can not be isolated from or detected in a water extract, but have been isolated from an extract made by treating the soil with a dilute solution of sodium hydroxide. This may be explained in several ways: First. The compounds are generated from some insoluble and probably more complex compounds by the action of the sodium hydroxide. This is the explanation that probably occurs first to those not familiar with the properties and antecedents of the compounds or with the general nature of the organic matter of soils. Second. The compounds may be present in the soil in insoluble form in combination with some of its mineral constituents, and these are soluble in alkaline solution and broken up by subsequent treatment with acid. This is probably the case with some of the organic acids isolated. Alumina in considerable quantity is always present in the sodium hydroxide extract, and every indication points to the presence in the soil of combinations between some of the organic acids and alumina and other inorganic bases. In such a case if the acid were one possible of being extracted from water solution with ether it would be found in the ether which had been shaken with the filtrate after acidifying the alkaline extract and filtering. Third. The compounds that under ordinary circumstances would be soluble in water are held in the soil in more or less insoluble form by being incorporated in granules or aggregates of insoluble material, such as resins or waxes. Resins and bodies of a similar nature make up a very large proportion of the soil organic matter soluble in alkalis. On treatment with sodium-hydroxide solution these resinous bodies go into solution, and the other organic compounds are set free, so to speak; that is, they are found in the filtrate when the resins are precipitated in a finely divided condition by acidifying. Whether there is a chemical combination between the alkali and the resins, a formation of sodium salts of resin acids, whether it is true solution or so-called colloidal solution, are questions, important of course, but questions whose settlement does not

effect the general statement just made. Fourth. The soluble organic compounds may be held absorbed by the insoluble finely divided organic matter. That this material has a great absorptive power is well known, and the fact that some organic compounds soluble in water can be obtained from the soil by continued leaching with water indicates that they are held there in this way.

Careful consideration of all the facts connected with the isolation of the organic compounds so far described must result in the elimination of the explanation first mentioned. There is no evidence that any of the compounds so far found in soils can be formed from other material by simple treatment with cold 2 per cent sodium hydroxide for a few hours. The general statement, however, that no change is or can be brought about by this treatment can not be entertained, for it is known that the character of protein is changed even by short contact with alkaline solution and that acids in small quantity are generated from sugars in the same way. This makes it necessary to decide in each individual case whether the particular compound isolated could have been generated from some other compound or compounds by any of the treatments involved in the methods used. For instance, the purine bases, xanthine, and hypoxanthine, are found as such in plants and animal tissues and in addition are formed by the hydrolysis of nucleic acids with strong mineral acids and heat. They are not known to be derived from any other source than nucleic acids and from these only by strong chemical reagents. The nucleic acids from which they are formed are quite resistant to the action of alkalis and there can be but one conclusion that when they are isolated from soil solution they exist as such in the soil. A similar line of argument applies to the hexone bases and the pyrimidine derivatives. In the case of organic acids the isolation of a free acid does not necessarily imply that this acid occurs free in the soil. It may, and probably in most cases does, occur there as a salt and is set free by the treatment to which the soil has been subjected. Without going into each case at this time it may be confidently stated that investigation will show that there is no evidence pointing to the generation of any of the organic compounds isolated from soils from other compounds of different constitution, and that such compounds occur as such in the soil, and in the case of acids either free or combined with bases.

UNIFICATION OF METHODS.

With the growing complexity of methods incident to the isolation of additional organic compounds from the soil the desirability of possible simplification or unification of these methods suggests itself. At present the experience of these working along this line supports the view that, once an organic compound has been found in soils and a reliable method devised for finding it, the surest way to determine

its presence or absence in other soils is to use a sample of the soil for a special search for that compound alone. There are two reasons for this: First. The methods used begin to diverge once an alkaline extract is obtained. An alkaline, neutral, or acid solution may be required, or if acid, it may be necessary to decide whether acetic, sulphuric, nitric, or some other acid should be used. For instance, starting with a sample as large as 50 pounds of soil, if an attempt be made to include a search for all compounds the extract will be divided and subdivided so often that the units will represent in many cases too small a portion of soil for the isolation of any compound. On the other hand, if the sample be made large enough to overcome this difficulty the volume of extract and the whole procedure would be beyond the limits of any laboratory equipment. Second. Different soils require different treatment, and knowledge of the peculiarities of any soil can only be obtained by working with it. It is seldom that an organic compound can not be separated from mixtures in more than one way, but it frequently happens that one way will be successful with one soil, while with another soil some modification must be introduced. The difference in methods that have to be resorted to in order to overcome difficulties presented may involve the kind of acid used, the concentration of the extract, the nature of the precipitant, the removal of one compound before another is sought, or the manipulation of the small crystalline residues obtained in the final stages.

While the best results in the isolation of organic compounds from soil will be obtained by working separate portions, probably not exceeding 50 pounds each, there are many points where compounds can be grouped together or where the extract from which one compound has been separated can be used for the isolation of others. For instance, the purine bases form a group, the hexone bases another, and the acid extract from which dihydroxystearic acid or trithiobenzaldehyde has been separated by ether can be used for other separations.

In carrying out the procedure in the methods described in the foregoing pages a number of unexpected difficulties have been encountered and some of these have occurred so often and in so many different places that some mention of them is warranted.

PRECIPITATION WITH METALLIC SALTS.

When a neutral soil extract obtained in the usual way by extracting with sodium hydroxide, neutralizing, and filtering, is treated with a metallic precipitant such as lead acetate, silver nitrate, mercuric nitrate, etc., the precipitate is usually voluminous and may contain a great variety of compounds. For instance, the precipitate

with silver nitrate might contain, of the compounds already isolated from soils, picoline carboxylic acid, dihydroxystearic acid, oxalic acid, saccharic acid, nucleic acid, and if slightly alkaline, purine bases, pyrimidine derivatives, and histidine, besides unidentified organic matter and some mineral matter. It is seldom that a soil extract will give a precipitate of any single compound with any of these metallic salts. This difficulty may be met in a variety of ways, such as fractional precipitation, separation of the residue obtained into fractions by treatment with solvents, ether, absolute alcohol, etc., or reprecipitation, either with the same precipitant or others.

PRECIPITATION WITH PHOSPHOTUNGSTIC ACID.

The precipitates formed by phosphotungstic acid in sulphuric acid solution, while usually not so voluminous as those produced by the metallic precipitants in neutral solution, like them, usually contain a medley of compounds. It can for this reason be used either for freeing a solution from material not wanted or as a preliminary precipitant followed by fractionation of the material by reprecipitation with other metallic salts. In decomposing the phosphotungstic acid precipitate the use of a mixture of 4 volumes of acetone and 3 of water, as suggested by Wechsler,¹ as a solvent for the precipitate before treatment with barium hydroxide was found an improvement over the procedure usually followed.

In the use of phosphotungstic acid as a precipitant it was sometimes found in carrying out the method ordinarily used that after the excess of barium had been removed by carbon dioxide there was still barium in solution, owing, probably, to the presence of some organic acid stronger than carbon dioxide. This barium could be removed by careful treatment with sulphuric acid and filtration from barium sulphate. The filtrate contained an unknown acid. If this acid should occur together with a base precipitated by phosphotungstic acid such as lysine, the identity of the final products might be difficult to determine.

CALCIUM SULPHATE CONTAMINATION.

Nearly all soils contain calcium and sulphates, so that under certain conditions gypsum crystals are deposited from the soil extracts. Under certain conditions calcium sulphate is precipitated or carried down, perhaps as a double salt, with metallic precipitants and on decomposing with hydrogen sulphide is found in the filtrate and crystallizes out in the residue. It is usually present when lead, silver, copper, or mercury precipitates are formed in slightly alkaline media. As the calcium sulphate crystals are often similar in appearance to the organic compounds sought, they may mislead and

¹ Z. physiol. Chem., 73, 138 (1911).

when, as often happens, their solubility is nearly the same as the organic compounds accompanying them, separation is troublesome. Recognition of the presence of calcium sulphate in such residues and repeated evaporations and extractions with absolute alcohol will often overcome this difficulty.

CALCAREOUS SOILS.

Calcareous soils yield often but a small portion of their organic matter when treated with sodium hydroxide solution unless previously leached free of lime with hydrochloric acid as in the ordinary humus determination. In some cases this is an advantage, since the large quantity of amorphous resins that make up so large a part of the humus often interferes with the separation of other organic compounds. The first isolation of histidine and arginine was from a dark-colored calcareous soil that on treatment with alkali yielded a light straw-colored extract from which these compounds were obtained. After removal of the lime with dilute acid this soil gave a very dark-colored alkaline extract. It has been thought that perhaps for the isolation of some compounds the addition of lime to non-calcerous soils before extraction with alkali would be advantageous, but this has not been thoroughly tested.

CHANGES IN ORGANIC COMPOUNDS.

It is quite evident that under ordinary field conditions there is going on a constant change and rearrangement of the organic compounds in the soil. Compounds added to the soil in vegetable or animal débris are being broken down into simpler compounds by the activity of enzymes and microorganisms, and the simpler compounds used as food by other forms of the microflora may be built up into other more complex compounds. It follows then that although certain compounds may be nearly always present, the quantitative relations are constantly changing. This change is not confined to soils in the field. Samples taken for examination and stored in the laboratory are also subject to change in the character of the organic compounds present. This has been found to be particularly true with regard to certain compounds. A soil from which dihydroxystearic acid had been isolated several times from separate portions after remaining in storage several months became so changed that but traces of this compound could be found. Observations covering a shorter time indicate that salicylic aldehyde and mannite tend also to disappear when the soils are stored. It follows then that if duplication of results be made a criterion in deciding the presence or absence of any organic compound in a soil, the duplicates should coincide in time of examination as well as method of treatment and source of sample.

CONCLUSION.

The organic compounds isolated from soils and described in this bulletin, together with those previously reported, make a list that may be classified as follows: Thirteen organic acids, nine organic basic compounds, three carbohydrates, two aldehydes, two alcohols, one each hydrocarbon, glyceride, ester, sulphur compound, phosphorus compound, and an acid hydride. This list viewed in the light of possible compounds is so far from complete as to be properly regarded but a beginning, still, when classified, it probably indicates roughly the proportions of the classes of organic compounds present in soils. Organic acids make up a large proportion of the organic matter in the soil and when the great variety of acids in plant tissues and the proportion of the organic matter of plants made up of this group of compounds is considered, as well as the fact that the other groups of compounds in both plants and animals yield acids on decomposition, this preponderance of organic acids in soils becomes a matter not only easily explained but one to be expected. There is no reason why any of the organic acids found in plants or found in the decomposition of plant or animal remains should not be found at some time in some soils. The great variety of organic acids possible from these sources, and their susceptibility to change under soil conditions tend to bring about an individuality of soils in respect to organic acid content much more pronounced than is possible with any other group of organic compounds. As has already been stated there is every indication that in most cases the organic acid isolated from soils is present in the soils as salts. In the case of acid soils no conclusive evidence has been obtained of the nature of the acid to which the acidity is due. It is worthy of note that dihydroxystearic acid, one that has been shown to be of rather common occurrence, has not been found in soils that were decidedly acid in reaction.

Organic bases that occur in plants or animals or arise in the decay of the same are comparatively few in number, and the nine so far isolated from soils probably make up half or more of those of likely or probable occurrence in soils. Organic bases as well as acids no doubt often are present in the soil in combination either with organic or mineral acids. The demonstration of the presence of organic bases in soils is of rather more than usual interest in view of the prevailing idea that soil organic matter is primarily acid, and further from the fact that these nitrogenous compounds have been shown to be directly assimilated by plants as well as to furnish much of the material from which nitric acid is generated in the process of nitrification.

The carbohydrates are represented by pentosans, apparently a constant constituent of soils, a glucoside containing rhamnose and mannite. These form but a very small part of the carbohydrate material that is added to soils and probably but a small part of that

which persists in the soil. The susceptibility of the carbohydrates, starches, and sugars, to decomposition by enzymes and microorganisms makes it likely, as has already been pointed out, that these plant constituents either disappear altogether or are represented by some of the products of decomposition, largely organic acids. Oxalic and saccharic acid probably have their origin in this way and there are no doubt many other acids not yet found that are derived in like manner.

Aldehyde compounds seem to be rather constant constituents of the soil organic matter. The salicylic aldehyde isolated from one soil is the only identified representative of this class. The unidentified aldehyde isolated or a similar body or mixture is found in nearly all soils.

Hydrocarbons represented by hentriacontane, one representative each of glycerides and resin esters, and alcohols represented by agosterol and phytosterol, are usually found in the humus precipitate formed on acidifying the alkaline extract of the soil. The present investigations do not include any examination of this material additional to that presented in previous bulletins.

The fact that there are many soils that give tests indicating the presence of organic sulphur compounds in which trithiobenzaldehyde can not be found indicates that there are other organic sulphur compounds in soils. The very definite way in which sulphur-containing proteins yield cystin on hydrolysis, the easy change of this to cystein on reduction and the further ready change of both these to thiolactic acids make it probable that some or perhaps all of these will ultimately be found in soils.

Phosphorus organic compounds are represented by nucleic acid, of which there are probably several. The only nucleic acids of plant origin that have been investigated to any extent are those from yeast and from the wheat embryo. These are said to be identical and it may be that the nucleic acids of plant origin do not present so great a variation in structure as do those of animal origin. It has been found that some nucleic acids under certain conditions split into compounds less complex than the original but more complex than the compounds usually considered as the end products, pentoses, bases, and phosphoric acid. Under certain conditions the split may result in free phosphoric acid and compounds known as nucleosides. The nucleosides are combinations of the pentose and purine base. Under other conditions the split may result in a compound known as a carbohydrate phosphoric acid. One of these compounds, *d* ribose phosphoric acid, $C_5H_{11}O_8$,¹ results from the decomposition of inosinic acid, while another, thymo-hexose-phosphoric acid, $C_{11}H_{17}U_2PO_{10}$,²

¹ Levene & Jacobs, Ber. deutsch Chem., 41, 2703 (1908).

² Levene & Mandel, Ber. deutsch Chem., 41, 1905 (1908).

results from the decomposition of thymus nucleic acid. There has been obtained some evidence of the presence of compounds of a similar nature in soils, but as yet no separation in a form definite enough for identification. There is also considerable evidence pointing to the presence in soils of both lecithin and phytin, the two other representatives of phosphorus organic compounds.

The general relationships between the different groups of organic compounds found in soils has been casually referred to under individual compounds, but can be more profitably presented in one statement.

• The organic acids, of which there are a great number in plants and probably a larger number in soils, may, when found in soils, be the result of direct addition of such acids in vegetable debris or of the decomposition and rearrangement of other organic matter that takes place in soils through the activity of microorganisms. The acids that originate through decomposition may be derived from almost all other classes of compounds. The breaking down of fats, glycerides, or protein, or the oxidation of carbohydrates, alcohols, aldehydes, or hydrocarbons, may furnish organic acids and these acids may be changed from one member to another of the same series or other series by the process of oxidation and reduction that accompany the biological activity of soils.

Many of the organic bases are rather intimately connected with protein, either those like histidine, arginine and lysine that result from the breaking down of the protein molecule or the purine bases, xanthine and hypoxanthine and adenine, or the pyrimidine derivative cytosin that results from the splitting of nucleic acid, itself a splitting product of the more complex nucleo-protein. The simpler bases, choline and trimethylamine, as has been mentioned, are decomposition products of lecithin. Lecithins are present in practically all growing cells both of plants and animals, and choline often occurs as such. Trimethylamine, while occurring as such in plants is much more apt to be found in decomposing organic material where no doubt it is derived from choline or the antecedent lecithin.

Carbohydrates are rather poorly represented among the compounds isolated from soil organic matter when the abundance of such material in vegetable remains is considered, but it seems probable that there are a number of the compounds of the group still undetected. The pentose sugars that are always formed on heating soils with mineral acids, may be formed from pentosans, from nucleic acids or from glucosides, and there is no method of determining their origin in any case except isolation of the parent body as was done with nucleic acid, pentosan, and the glucoside, which yielded rhamnose.

With regard to the character of organic sulphur compounds in the soil the investigation at the present time leaves much to be desired. The one compound isolated is apparently not a normal

constituent of soils, and as has already been noted, it is difficult to see any relation between it and the sulphur compounds known to occur in plants or to result from their decomposition.

The isolation of nucleic acid from soils, while by no means the last word on the question of the organic phosphorus in soils, at least settles the question whether there are organic phosphorus compounds in soils. The isolation and identification of nucleic acid from a number of soils leaves no longer any doubt in this regard. The common occurrence of the purine base, hypoxanthine, the occasional occurrence of xanthine and adenine and of cytosine is, of course, intimately related to the presence of nucleic acid, as is also a portion of the pentose sugar obtained on hydrolysis.

Much of the discussion found in agricultural literature regarding organic phosphorus compounds in soils has centered around methods for distinguishing between organic and inorganic phosphorus. Some of the discussion has been futile from lack of recognition of the presence of nucleic acids and due consideration of their properties. They are often very easily decomposed by the action of mineral acids, even when dilute and at ordinary temperatures. When a soil is extracted with dilute alkali, the alkaline extract contains nucleic acid as well as probably some inorganic phosphorus compounds. When the solution is acidified with a mineral acid and the precipitate washed with dilute acid usually very little phosphorus is left in the so-called humus precipitate. For instance, a soil containing 0.316 per cent phosphorus gave an extract with 2 per cent sodium hydroxide, which contained 0.185 per cent phosphorus, calculated on the soil. On acidifying this extract and washing the precipitate, this contained only 0.0006 per cent phosphorus. The acid filtrate from this precipitate then contains all but a trace of the phosphorus originally extracted by the sodium hydroxide. This may be the original nucleic acid and the inorganic phosphorus in the original extract, but if the procedure has been of some duration the nucleic acid is decomposed, free phosphoric acid resulting. If care is taken to perform this operation in a short time, nucleic acid can be recovered from the acid filtrate by adding sodium acetate and several volumes of alcohol, but the quantity obtained will be decreased by several hours' standing, as well as by an excess of acid, and may disappear altogether in a few days. Individual soils differ markedly in this respect. The differentiation between the phosphoric acid combined in nucleic acid and free phosphoric acid is experimentally so difficult that any attempt to determine quantitative relationships between them is at present futile. When, by the procedure just outlined, nucleic acid is obtained by exercising all care to prevent decomposition, and when by standing some hours less is obtained, there is still no means of ascertaining whether the quantity of nucleic acid

obtained in the first instance represented all that was extracted from the soil. In other words, there is no standard to judge by and with all precautions some free phosphoric acid may be generated from the nucleic acid.

In this connection it may be well to mention another point that has to do with both organic phosphorus and sulphur compounds. This is the quite different characters of the two classes of compounds. Nucleic acids and lecithins may be regarded as substituted phosphoric acids, the phosphorus being, so to speak, in the phosphoric acid form, and phosphoric acid as such being split off on the least provocation. The same is probably true of phytin, although its constitution is not so well established. In organic sulphur compounds on the other hand, the sulphur may usually be regarded as in the place of oxygen, for instance, in trithiobenzaldehyde there is the group CSH in the place of COH. When such compounds split, free sulphur or a sulphide usually results, few, if any, having sulphur in such form as to split off sulphuric acid, viz, sulphur in combination with oxygen. There is then no analogy between the relation of organic phosphorus compounds and phosphoric acid on the one hand and organic sulphur compounds and sulphuric acid on the other.

As was stated in the introduction there was in carrying out this work no attempt made to include all the organic matter in any one soil in the investigation. The aim was rather to isolate compounds not heretofore found in soils with the hope, not only of adding to the knowledge of the organic matter of soils in general, but also of throwing light on such abnormal characteristics as some particular soil presented. For this reason it is not possible to make any specific or even general statement as to what properties of the soil organic matter the 15 compounds described in this bulletin represent.

There seems to be an idea prevalent in some quarters that the organic chemical compounds shown to be present in the soil organic matter are rather incidental contaminations of the larger, more important, and "still mysterious" humus. Now while it is true that there still remains a large proportion of the organic matter unidentified, it is not so large in the soil of average organic content as many imagine. This unidentified portion is now, known to be made up largely of compounds related to the resins, and the identification of its constituents is difficult because the chemistry of this class of plant constituents is still in a chaotic condition, and there is almost nothing definite with which to compare an isolated soil resin or resin acid.

The compounds described are all extracted from soil by dilute alkali and are a part of the organic matter included in the general term humus, but they are for the most part not precipitated when this extract is acidified. In other words, they are a part of the humus extract, but not included in the "matiere noir" except as held in it by

absorption. Except in the case of soils high in humus content, this portion of the organic matter of soils is one-half or more of that extracted by alkalis and with the knowledge of the compounds present in this fraction and the methods available for their isolation or determination it is in many cases possible to account for nearly the whole of it. The work here reported bears out a conclusion reached in summing up previous work that while it emphasizes the complex character of the organic matter of soils, it demonstrates at the same time that this complexity is not so great that the chemical nature of all of the organic soil material can not be determined by modern methods of research.

SUMMARY.

In this bulletin there is presented the result of further research into the chemical nature of the organic matter of soils. The isolation of 15 organic compounds from this material is described, 14 of which have been identified. These together with those isolated before and described in previous bulletins make 35 organic compounds isolated from soils.

The methods by which these compounds were isolated are described, the manner in which they have been identified pointed out, and the possible sources of the compounds discussed. The substances here described belong to 6 classes of organic compounds, 3 classes, aldehydes, organic sulphur, and organic phosphorus compounds being classes not represented among those isolated and described before.

The compounds isolated have been classified as follows: Thirteen organic acids, 9 organic bases, 3 sugars, 2 aldehydes, 2 alcohols, and 1 each, hydrocarbon, glyceride, resin ester, sulphur compound, phosphorus compound, and an acid anhydride.

The relationship between these groups as well as that between the isolated compounds and the organic matter in general is discussed.

The compounds isolated and described here are oxalic acid, $C_2H_2O_4$; succinic acid, $C_4H_6O_4$; saccharic acid, $C_6H_8O_{10}$; acrylic acid, $C_3H_4O_2$; lysine, $C_6H_{14}O_2N_2$; adenine, $C_5H_5N_5$; choline, $C_5H_{15}O_2N$; trimethylamine, C_3H_9N ; salicylic aldehyde, $C_6H_4OH_1COH$; mannite, $C_6H_{14}O_6$; rhamnose, $C_6H_{14}O_{10}$; trithiobenzaldehyde, $(C_6H_5CSH)_3$; nucleic acid, of unknown constitution, and an unidentified aldehyde.

The conclusion is reached that the work, like that previously reported, while it emphasizes the complex character of the organic matter of soils, bears out the contention that this complexity is not so great but that the chemical nature of all of the organic matter of soils can be determined by modern methods of research.

